

REPLACEMENT SHEET 1 / 9

Construct of Feline Thyrotropin-beta Subunit with First Intron

1 30
 5'(GAA TTC)ATG ACT GCT ATC TAC CTG ATG TCC GTG CTT
 met thr ala ile tyr leu met ser val leu

31 75
 TTT GGC CTG GCA TGT GGA CAA GCG ATG TCT TTT TGT TTT CCA ACT
 phe gly leu ala cys gly gln ala met ser phe cys phe pro thr
 76 120
 GAG TAT ATG ATG CAT GTC GAA AGG AAA GAG TGT GCT TAT TGC CTA
 glu cys met met his val glu arg lys glu cys ala tyr cys leu
 121 162
 ACC ATC AAC ACC ACC ATC TGT GCT GGA TAT TGT ATG ACA CGG
 thr ile asn thr thr ile cys ala gly tyr cys met thr arg

Intron 1
 163 ***GTATGTAGTTCATCTCACTTCTTTTAGCTGAAAATTAGATAAACCTAGACT***
CAGTCCATTTCTATCCAGAAAGGAAATGAGATAAATCACAACCTCATTTACAGACCTAAC
GGTCATTGGCTCCTTAGAGGTAGAGTCCCTAGGTTATAATATACGGACCTACTCCATACAG
TTGGTACAGATAATTTTACAATAGTTTTACTCCCAAAGTTTATTTAAACCTTATCTTGTTCC
CACGATCAAGGATAAAAAGAGAGGTGTGTGTGTATGTCATTTTTTTTTTGTCTCTATAGGATT
CAGTGTGGATATGCTGAATTGGTATTGGGGAATGGGACTAAGGAATCCTCCCCCAGTCCTA
TTTGTATCTATGGGATGTAAGCGAATTAACATTTTGCTTCCTCTTCTGTGCTTCCCTCAG
 580
 581 625
 GAT ATC AAT GGC AAA CTG TTT CTT CCC AAA TAT GCT CTG TCC CAA
 asp ile asn gly lys leu phe leu pro lys tyr ala leu ser gln
 626 670
 GAT GTT TGC ACC TAC AGA GAC TTC CTG TAC AAG ACT GTA GAA ATA
 asp val cys thr tyr arg asp phe leu tyr lys thr val glu ile
 671 715
 CCA GGA TGC CCA CAC CAT GTT ACT CCC TAT TTC TCC TAC CCG GTA
 pro gly cys pro his his val thr pro tyr phe ser tyr pro val
 716 760
 GCT GTA AGC TGT AAA TGT GGC AAG TGT AAT ACT GAC TAT AGC GAC
 ala val ser cys lys cys gly lys cys asn thr asp tyr ser asp
 761 805
 TGC ATA CAT GAG GCC ATC AAG ACA AAT GAT TGT ACC AAA CCC CAG
 cys ile his glu ala ile lys thr asn asp cys thr lys pro gln
 806 835
 AAG TCC GAT GTG GTA GGA GTT TCT ATC TAA (GCGGCCGC₍₄₎)(AT)₅-3'
 lys ser asp val val gly val ser ile stop

() denotes the Eco RI restriction sites

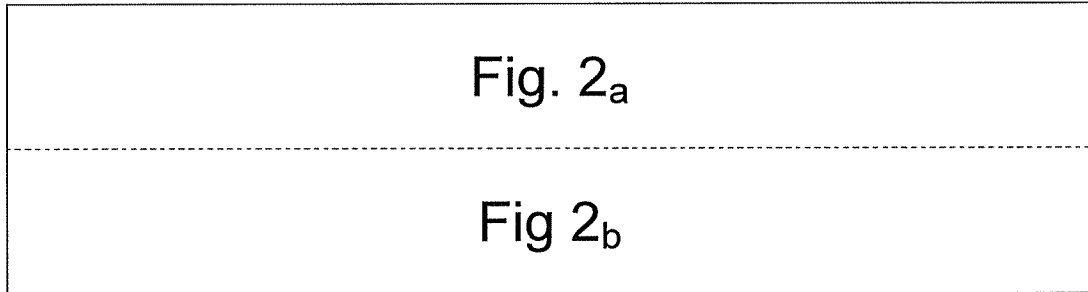
Bold denotes signal sequence

Bold/italic denotes the intron 1 sequence

Fig. 1

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Feline Thyrotropin Alpha Subunit Construct



Bold denotes 24 amino acid unexpressed signal sequence as per structure in other species
Bold italics denotes sequence upstream from expressed but not secreted signal sequence;
only reported in equine; whether it is expressed is not clear.

() denotes Eco R1 restriction site from TOPO Blunt vector

Underlined denotes additional sequence from TOPO Blunt vector

((1)) denotes Factor XA site

((2)) denotes Flag tag

((3)) denotes stop codon

((4)) denotes NotI restriction enzyme site

()₅ denotes extra bases needed for restriction enzyme to work

Fig. 2

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(GAATTC) GCCCTT

1 45
AGT TAC TGA GAA ATC ACA AGA CGA AGC CAA AAT CCC TCT TCA GAT
ser tyr OPA glu ile thr arg arg ser gln asn pro ser ser asp
46 90
CCA CGG TCA ACT GCC CTG ATC ACA TCC TGC AAA AAG TCC GGA GGA
pro arg ser thr ala leu ile thr ser cys lys lys ser gly gly
91 135
AGG AGA GCC ATG GAT TAC TAC AGA AAA TAT GCA GCT GTC ATT CTG
arg arg ala met asp tyr tyr arg lys tyr ala ala val ile leu
136 180
GCC ATA CTC TCT GTG TTT CTG CAT ATT CTC CAT TCT TTT CCT GAT
ala ile leu ser val phe leu his ile leu his ser phe pro asp
181 225
GGA GAG TTT ACA ATG CAG GGG TGC CCA GAA TGC AAG CTA AAG GAA
gly glu phe thr met gln gly cys pro glu cys lys leu lys glu
226 270
AAC AAA TAC TTC TCC AAG TTG GGT GCC CCA ATT TAT CAA TGC ATG
Asn lys tyr phe ser lys leu gly ala pro ile tyr gln cys met
271 315
GGC TGC TGC TTC TCC AGA GCA TAC CCC ACT CCA GCA AGG TCC AAG
gly cys cys phe ser arg ala tyr pro thr pro ala arg ser lys
316 360
AAG ACA ATG TTG GTC CCA AAG AAC ATC ACC TCA GAA GCC ACA TGC
lys thr met leu val pro lys asn ile thr ser glu ala thr cys
361 405
TGT GTG GCC AAA GCC TTT ACC AAG GCC ACG GTA ATG GGA AAT GCC
cys val ala lys ala phe thr lys ala thr val met gly asn ala

Fig. 2_a

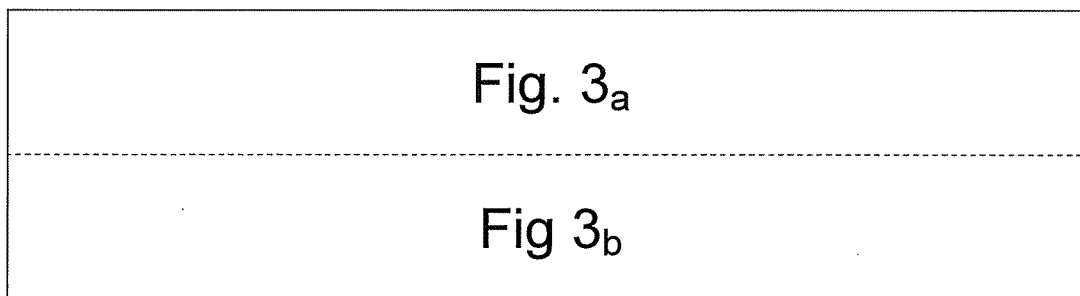
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406 450
AAA GTG GAG AAT CAC ACA GAG TGC CAC TGC AGC ACT TGC TAT CAC
lys val glu asn his thr glu cys his cys ser thr cys tyr his
451 459 492
CAC AAG ATT (ATC GAA GGT CGT₍₁₎)(GAC TAC AAG GAC GAT GAC GAT
his lys ile ile glu gly arg asp tyr lys asp asp asp asp
493 495 510
AAG₍₂₎ (TAA₍₃₎) (GCGGCCGC₍₄₎)(TATG)₅ 3'
lys

Fig. 2_b

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Yoked Feline Thyrotropin



- () denotes the Eco RI restriction sites
Bold denotes signal sequence
Bold italics denotes intron 1 sequence 1=Factor XA site
((1)) denotes Factor XA site
((2)) denotes Flag tag
((3)) denotes stop codon
((4)) denotes NotI restriction enzyme site
()₅ denotes extra bases needed for restriction enzyme to work

Fig. 3

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1 30
5'(GAA TTC)ATG ACT GCT ATC TAC CTG ATG TCC GTG CTT
met thr ala ile tyr leu met ser val leu

31 75
TTT GGC CTG GCA TGT GGA CAA GCG ATG TCT TTT TGT TTT CCA ACT
phe gly leu ala cys gly gln ala met ser phe cys phe pro thr

76 120
GAG TAT ATG ATG CAT GTC GAA AGG AAA GAG TGT GCT TAT TGC CTA
glu cys met met his val glu arg lys glu cys ala tyr cys leu

121 162
ACC ATC AAC ACC ACC ATC TGT GCT GGA TAT TGT ATG ACA CGG
thr ile asn thr thr ile cys ala gly tyr cys met thr arg

Intron 1

163 GTATGTAGTTCATCTCACTTCTTTTAGCTGAAAATTAGATAAACCTAGACT
CAGTCCATTTCTATCCAGAAAGGAAATGAGATAAATCACAACCTCATTTTCACAG
ACCTAACGGTCATTGGCTCCTTAGAGGTAGAGTCCCTAGGTTATAATATACGGA
CCTACTCCATACAGTTGGTACAGATAATTTTTACAATAGTTTTACTCCCAAAGTT
TATTTAAACCTTATCTTGTTCCCACGATCAAGGATAAAAGAGAGGTGTGTGTGT
ATGTCATTTTTTTTTGTCTCTATAGGATTCAGTGTTGGATATGCTGAATTGGTATT
GGGGAATGGGACTAAGGAATCCTCCCCCAGTCCTATTTGTATCTATGGGATGT
AAGCGAATTAACATTTTGCTTCCTCTTCTGTGCTTCCCTCAG 580

581 625
GAT ATC AAT GGC AAA CTG TTT CTT CCC AAA TAT GCT CTG TCC CAA
asp ile asn gly lys leu phe leu pro lys tyr ala leu ser gln

626 670
GAT GTT TGC ACC TAC AGA GAC TTC CTG TAC AAG ACT GTA GAA ATA
asp val cys thr tyr arg asp phe leu tyr lys thr val glu ile

671 715
CCA GGA TGC CCA CAC CAT GTT ACT CCC TAT TTC TCC TAC CCG GTA
pro gly cys pro his his val thr pro tyr phe ser tyr pro val

716 760
GCT GTA AGC TGT AAA TGT GGC AAG TGT AAT ACT GAC TAT AGC GAC
ala val ser cys lys cys gly lys cys asn thr asp tyr ser asp

761 805
TGC ATA CAT GAG GCC ATC AAG ACA AAT GAT TGT ACC AAA CCC CAG
cys ile his glu ala ile lys thr asn asp cys thr lys pro gln

806 beta-specific primer sequence * CTP linker 850
AAG TCC GAT GTG GTA GGA GTT TCT ATC CAG GAC TCC TCT TCC TCA
lys ser asp val val gly val ser ile gln asp ser ser ser ser

851 CTP linker 892
AAG GCC CCT TCC GCC AGC CTT CCA AGC CCA ACG CGT CTC CCG
lys ala pro ser ala ser leu pro ser pro thr arg leu pro

* reverse complement in construct

Afl III ligation site

Fig. 3_a

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| 893 | CTP linker | alpha-specific primer sequence | 937 | * |
|---|------------|-----------------------------------|-----|---|
| GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA | | TTT CCT GAT GGA GAG | | |
| gly pro ser asp thr pro ile ile pro gln phe pro asp gly glu | | | | |
| 938 | | 977 | | |
| TTT ACA ATG CAG GGG TGC CCA GAA TGC AAG CTA AAG GAA | | | | |
| phe thr met gln gly cys pro glu cys lys leu lys glu | | | | |
| 978 | | 1022 | | |
| AAC AAA TAC TTC TCC AAG TTG GGT GCC CCA ATT TAT CAA TGC ATG | | | | |
| Asn lys tyr phe ser lys leu gly ala pro ile tyr gln cys met | | | | |
| 1023 | | 1067 | | |
| GGC TGC TGC TTC TCC AGA GCA TAC CCC ACT CCA GCA AGG TCC AAG | | | | |
| gly cys cys phe ser arg ala tyr pro thr pro ala arg ser lys | | | | |
| 1068 | | 1112 | | |
| AAG ACA ATG TTG GTC CCA AAG AAC ATC ACC TCA GAA GCC ACA TGC | | | | |
| lys thr met leu val pro lys asn ile thr ser glu ala thr cys | | | | |
| 1113 | | 1157 | | |
| TGT GTG GCC AAA GCC TTT ACC AAG GCC ACG GTA ATG GGA AAT GCC | | | | |
| cys val ala lys ala phe thr lys ala thr val met gly asn ala | | | | |
| 1158 | | 1202 | | |
| AAA GTG GAG AAT CAC ACA GAG TGC CAC TGC AGC ACT TGC TAT CAC | | | | |
| lys val glu asn his thr glu cys his cys ser thr cys tyr his | | | | |
| 1203 | 1211 | | | |
| CAC AAG ATT (ATC GAA GGT CGT ₍₁₎)(GAC TAC AAG GAC GAT GAC GAT | | | | |
| his lys ile ile glu gly arg asp tyr lys asp asp asp asp | | | | |
| 1245 | 1247 | 1262 | | |
| AAG ₍₂₎ (TAA ₍₃₎) (GCGGCCGC ₍₄₎)(TATG) ₅ 3' | | | | |
| lys | | | | |

* as written

Fig. 3_b

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PEAK Expression Vector (with yoked fTSH)

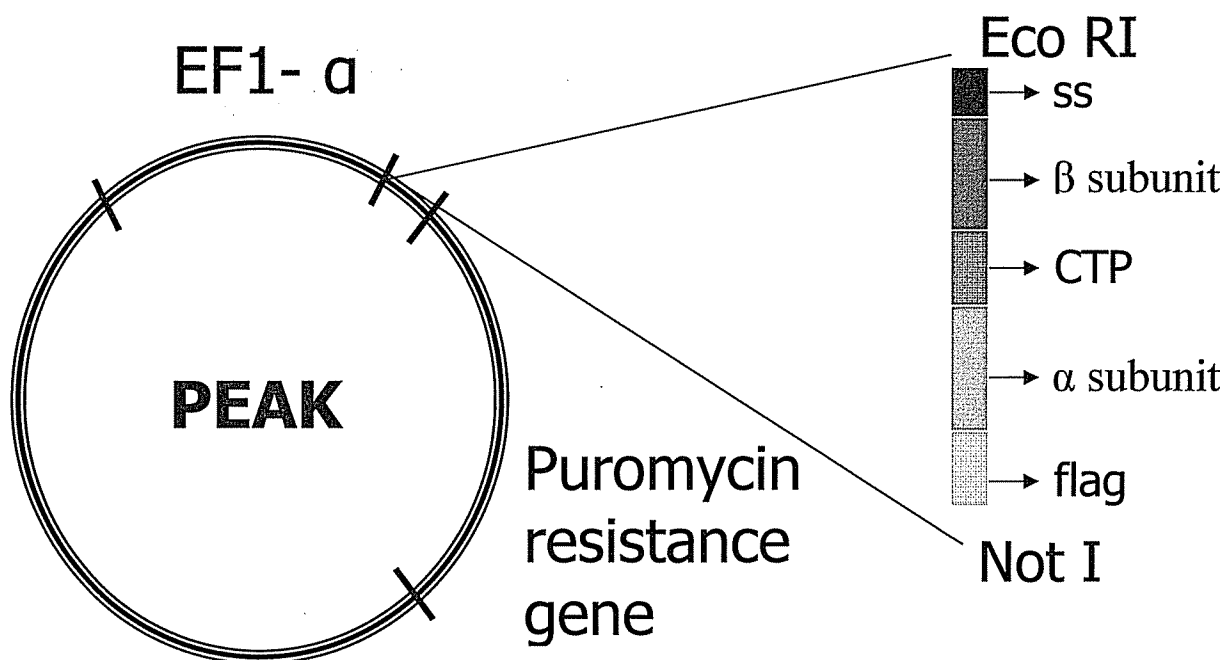


Fig. 4

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Fig. 5a

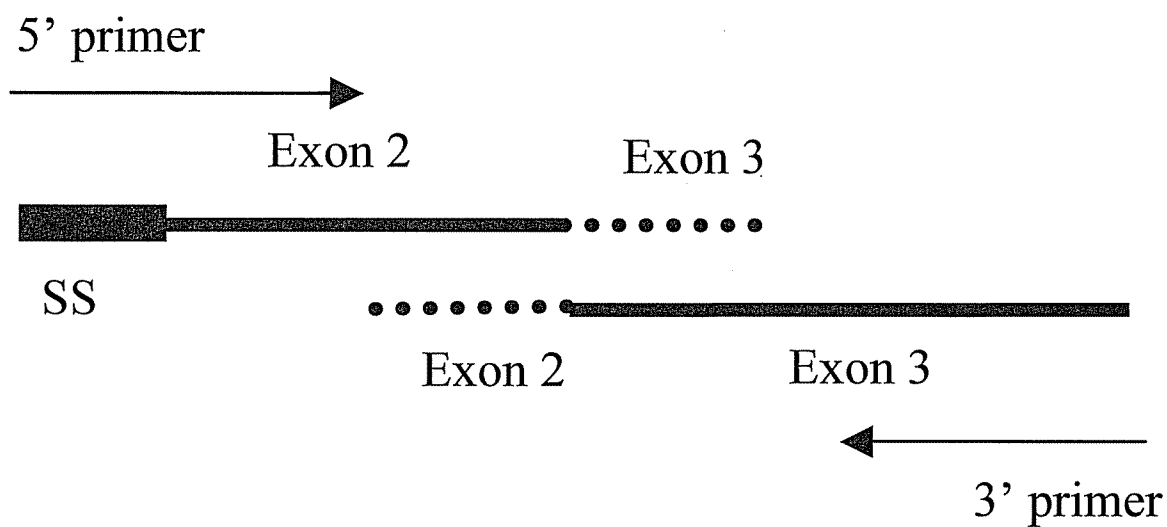


Fig. 5b

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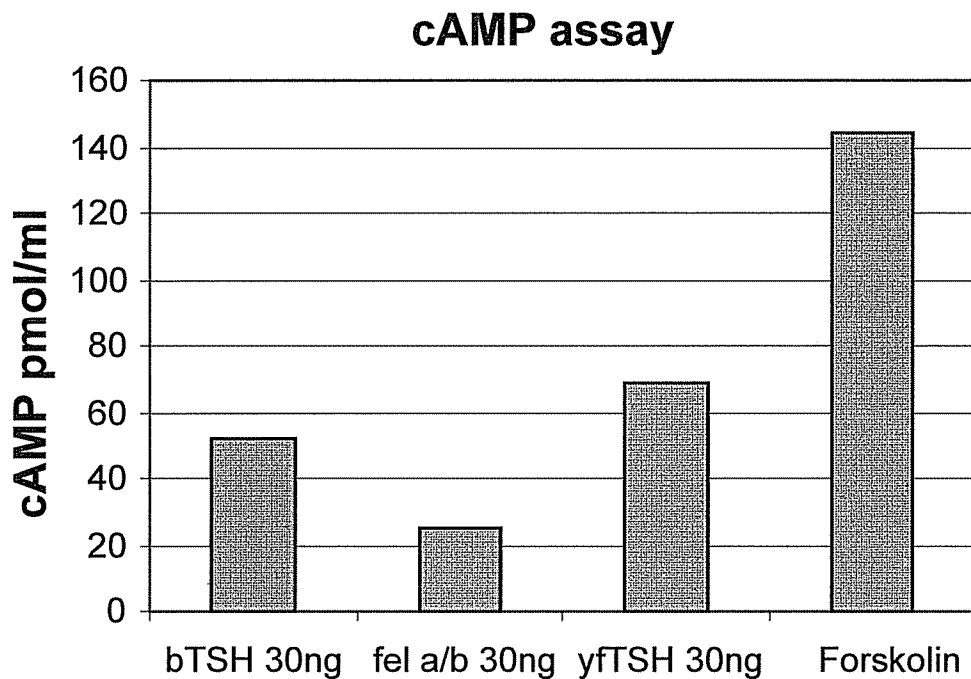


Fig. 6a

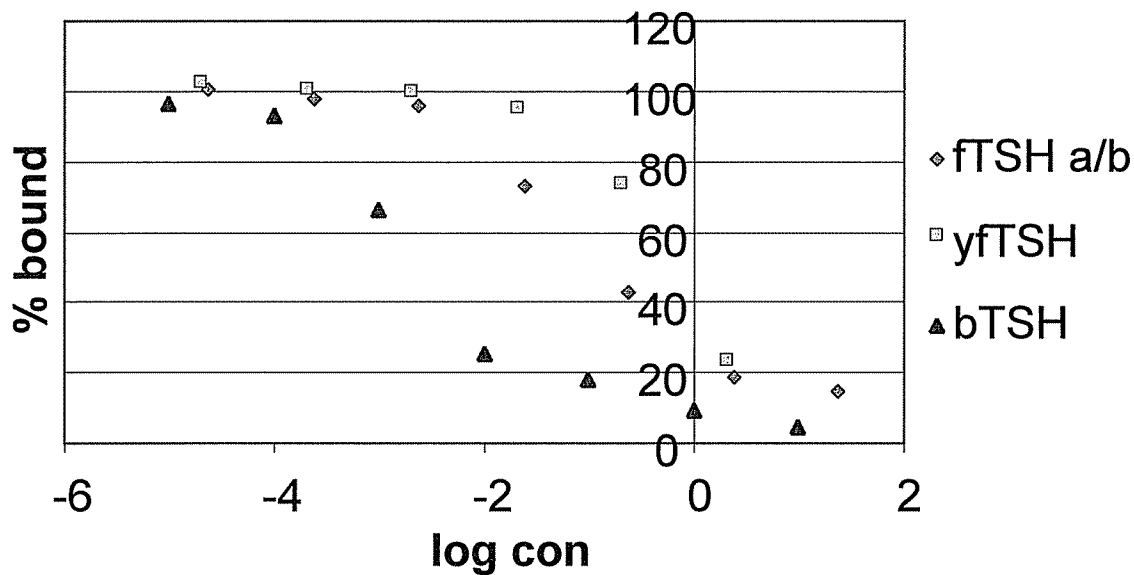


Fig. 6b